



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A61K 31/435</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 97/01336</b> <b>(43) International Publication Date:</b> 16 January 1997 (16.01.97)
<b>(21) International Application Number:</b> PCT/IB96/00742 <b>(22) International Filing Date:</b> 26 June 1996 (26.06.96) <b>(30) Priority Data:</b> 08/496,465      29 June 1995 (29.06.95)      US <b>(71) Applicant:</b> PHARMA MAR, S.A. [ES/ES]; Calle de la Calera, 3, E-28760 Madrid (ES). <b>(72) Inventors:</b> PUENTES, Jose Luis Fernandez; Calle Covadonga, 2, E-24004 Leon (ES). GARCIA GRAVALOS, Delores; Calle Maldonado, 63, E-28006 Madrid (ES). RODRIGUEZ QUESADA, Ana; De San Francisco, 8, Portal 6, 20, E-29008 Malaga (ES).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> USE OF LAMELLARIN-CLASS ALKALOIDS IN METHODS OF TREATMENT  <b>(57) Abstract</b>  The lamellarin compounds disclosed herein have been found to be inhibitors of MDR, i.e., acquired multidrug resistance, which has become a major problem in the treatment of various cancers. The lamellarin compounds disclosed herein have also been found to be cytotoxic to MDR cells. MDR is believed to be associated with certain alterations in tumor cells, including an over-expression of a certain high molecular weight membrane glycoprotein and a decrease in the ability of the tumor cell to accumulate and retain chemotherapeutic agents. The present invention is thus directed to methods of treating MDR-type tumors with an effective anti-MDR amount (either inhibitory or cytotoxic) of one or more lamellarin compounds, which compounds have been found to be effective antitumoral agents against MDR cells.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

- 1 -

## USE OF LAMELLARIN -CLASS ALKALOIDS IN METHODS OF TREATMENT

## BACKGROUND OF THE INVENTION

Marine ascidians have proven to be rich sources of structurally diverse alkaloids, many of which exhibit a broad spectrum of biological activity. Members from the family Didemnidae are generally highly colored, encrusting organisms, colonial by habit, and characteristically contain chemical constituents which are derived from amino acids. The polyaromatic lamellarin alkaloids, for example, are probably derived from three tyrosine residues. The lamellarin skeleton was first identified in an isolate from a prosobranch mollusc, *Lamellaria sp.*, from Palau, but has more recently been found in the didemnid ascidian *Didemnum chartaceum* from the Seychelles. It has also been suggested that the lamellarins may be distantly related to the tunichromes, reducing blood pigments isolated from the ascidian *Ascidia nigra*.

Anderson et al., *J. Am. Chem. Soc.*, 107: 5492-5495 (1985), describe the isolation and characterization of four polyaromatic metabolites, the lamellarins A-D, obtained from a marine prosobranch mollusc, *Lamellaria sp.* The structure of lamellarin A was determined by an X-ray crystallographic study and the structures of lamellarins B-D were assigned by interpretation of spectral data. The disclosure of this publication is hereby incorporated herein by reference.

- 2 -

Lindquist et al., *J. Org. Chem.*, 53: 4570-4574 (1988) describes the isolation and characterization of four new alkaloids of the lamellarin class from the marine ascidian *Didemnum chartaceum* obtained from the Indian Ocean. The structure of lamellarin E was determined by spectroscopic and X-ray crystallographic methods. The structure of lamellarins F-H were elucidated by interpretation of NMR spectral data. The disclosure of this publication is hereby incorporated herein by reference.

Carroll et al., *Aust. J. Chem.*, 46: 489-501 (1993) describes six new polyaromatic alkaloids, the lamellarins I, J, K, L, M and the triacetate of lamellarin N, and four known alkaloids of this type, lamellarin A, B, C and the triacetate of lamellarin D, isolated from a marine ascidian *Didemnum sp.* The disclosure of this publication is hereby incorporated herein by reference.

The lamellarin compounds disclosed herein have been found to be non-toxic inhibitors of acquired multidrug resistance (MDR), which has become a major problem in the treatment of various human tumors. The lamellarin compounds have also been found to be cytotoxic to MDR cells. Both of these activities are useful in the treatment of MDR tumors.

Drugs of proven antitumor chemotherapeutic value to which multidrug resistance has been observed include vinblastine, vincristine, etoposide, teniposide, doxorubicin (adriamycin), daunorubicin, plicamycin (mithramycin) and actinomycin D. Many tumors are intrinsically multidrug resistant (e.g., adenocarcinomas of the colon and kidney) while other tumors acquire multidrug-resistance during therapy (e.g., neuroblastomas and childhood leukemias).

- 3 -

While not wishing to be bound by theory, it is believed that the *mdr* gene encodes a glycoprotein (P-170 or P-glycoprotein). This protein is thought to act as an energy dependent efflux pump that is utilized in normal cells, as well as in cancer cells, for their detoxification. But, when the latter are able to over express the gene, the effect of an antitumor drug in such cells is highly reduced and therefore the MDR phenotype appears. See, for instance, Deuchars et al., *Seminars in Oncology*, **16**: 156-165 (1989) and Gottesman et al., *Ann. Rev. Biochem.*, **62**: 385-427 (1993). Two ways to overcome MDR are (1) find inhibitors of P-170 or (2) find drugs which are as active against the MDR cancer cell line as they are to said cell line's normal counterpart.

MDR inhibitors are agents that are used to restore drug sensitivity to some multidrug resistant tumor cells. Among the agents known to possess this property are certain calcium transport blockers (e.g., verapamil) and certain calmodulin inhibitors (e.g., trifluoperazine). However, clinical use of these compounds has been limited by their toxic side effects. See, Ozols et al., *J. Clin. Oncol.*, **5**: 541-547 (1987); see also, Twentyman et al., *Int. J. Radiat. Oncol. Biol. Phys.*, **12**: 1355 (1986). The minimization (or elimination) of such toxic side effects is thus an important factor in the selection of a MDR inhibitor.

Since verapamil was firstly described, several natural product compounds have been reported to overcome or inhibit MDR. Examples include the plant alkaloid thailblastine (see, Chen et al., *Cancer Res.*, **53**: 2544-2547 (1993)), and the marine natural product patellamine D (see, Williams et al., *Cancer Letters*, **71**: 97-102 (1993)). Other examples of compounds active against MDR cells are the peptide cyclosporin A (see, Beck et al., *Biochem. Pharmacol.*, **43**: 89-93 (1992)), a heterocyclic compound (5-N-acetylardeemin - see, Karwowsky et al., *J. Antibiotics*,

- 4 -

46: 374-379 (1993)), Geodiamolide A, jaspamide, and glaciasterol A (see, Stingi et al., *Cancer Chemother. Pharmacol.*, 30: 401-406 (1992)). Thus, the search for new MDR inhibitors and compounds active against MDR cells continues.

5

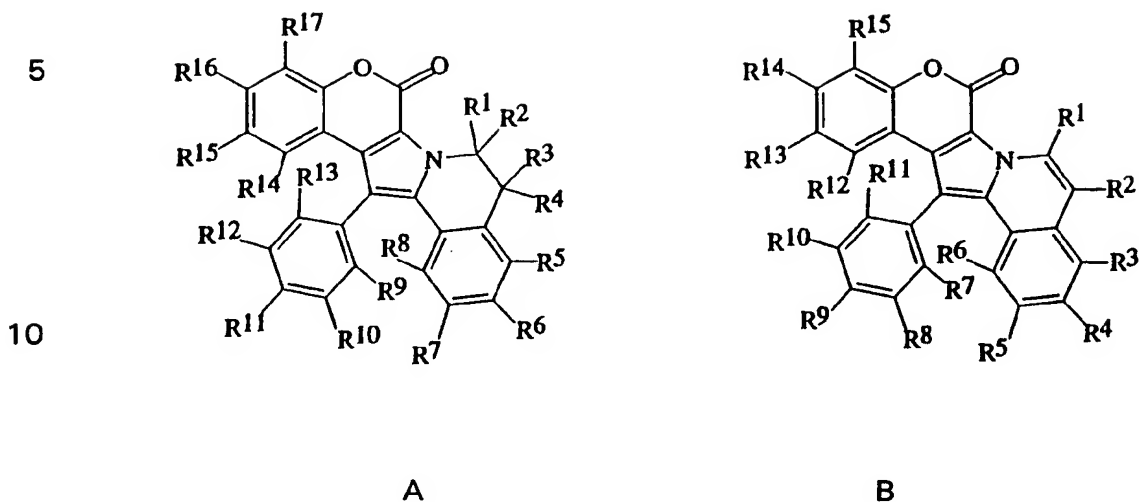
### SUMMARY OF THE INVENTION

The lamellarin compounds disclosed herein have been found to be non-toxic inhibitors of acquired multidrug resistance (MDR), which has become a major problem in the treatment of various human tumors. The lamellarin compounds have also been found to be cytotoxic to MDR cells. Both of these activities are useful in the treatment of MDR tumors. As discussed above, MDR is believed to be associated with certain alterations in tumor cells, including an over-expression of a certain high molecular weight membrane glycoprotein and a decrease in the ability of the tumor cell to accumulate and retain chemotherapeutic agents.

The present invention is thus directed to methods of treating selected tumors with an effective anti-MDR amount, i.e., either an inhibitory amount, a cytotoxic amount, or both, of one or more lamellarin compounds, which compounds have been found to be effective antitumoral agents against MDR cells.

Thus, in one preferred embodiment of the present invention, there is provided a method of treating (i.e., by slowing the growth or arresting the growth) MDR tumors comprising administration of an effective MDR inhibitory amount of a compound having the one (or both) of the following formulae:

30



15 wherein R<sup>1</sup> to R<sup>17</sup> (in Formula A) or R<sup>1</sup> to R<sup>15</sup> (in Formula B) may be the same or may be different and are selected from the group consisting of -H, -OH, -Me, -Et, -Pro, -OMe, -COMe, and -OCOMe.

20 Thus, in another preferred embodiment of the present invention, there is provided a method of treating (i.e., by slowing the growth or stopping the growth) MDR tumors comprising administration of an effective MDR cell cytotoxic amount of an anti-MDR lamellarin compound, particularly of Formula A or B as shown above.

25 Thus, the present invention is directed to methods of treatment using the lamellarin compounds embraced in the two general Formulas A and B, and more particularly to methods of treatment utilizing the lamellarins shown below Table I as especially preferred examples of compounds useful in the presently claimed invention.





- 7 -

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

As described above, the present invention is directed to novel methods of treating tumors in mammals, comprising administering to a patient in need of such treatment, lamellarins as either inhibitors of MDR activity, or as MDR cytotoxic compounds. Thus, the lamellarins may be employed either alone against MDR tumors, or in combination with other anti-tumor drugs, as effective treatments against MDR cells.

The present invention is also directed to pharmaceutical compositions comprising one or more lamellarin compounds useful as specified herein. Moreover, such pharmaceutical compositions can further comprise one or more other antitumor drug, particularly those in which multidrug resistance has been observed; including for example vinblastine, vincristine, etoposide, teniposide, doxorubicin (adriamycin), daunorubicin, plicamycin (mithramycin) and actinomycin D.

Thus, the present invention is also directed to a method of improving the antitumor chemotherapeutic effect of MDR affected drugs in patients in need of such treatment, comprising coadministering (simultaneously or sequentially) the MDR affected antitumor drug with an effective anti-MDR effective amount of a lamellarin compound.

As demonstrated herein, the compounds of the present invention have been found to possess MDR antitumor activity both *in vitro* and *in vivo*, and as such it is believed that these cytotoxic compounds will be useful as MDR antitumor compounds in animals and preferably in humans.

When being used as cytotoxic or antitumor inhibitory MDR agents,

- 8 -

the compounds of the present invention can be prepared and administered in various dosage forms, especially parenteral dosage forms. It will be clear to those having ordinary skill in this art that the dosage forms may comprise as the active ingredient, one or more of the compounds of the present invention. The skilled artisan will likewise recognize that the dosages and routes of administration will vary according to the needs of the patient and the specific activity of the active ingredient(s). The determination of these parameters is within the ordinary skill of the practicing physician.

Tables II and III below provide additional data regarding the MDR inhibitory activity of the lamellarins. These data were generated by the procedures described below.

TABLE II

LAMELLARINS TOXICITY TO SENSITIVE AND RESISTANT TUMOR CELL LINES
---

LAMELLARIN	STANDARD	ACTIVITY: IC <sub>50</sub> (μg/ml)			
		Mouse Lymphoma		Chinese Hamster Ovary	
		P-388	P-388, SCHABEL (MDR)	CHOB1	CHOC5 (MDR)
A		0.5	0.5	0.2	0.4
B		5.5	5.5	3.0	10.0
D-triacetate		0.07	0.09	0.03	0.04
I		2.5	2.5	0.2	1.0
I-acetate		5.5	5.5	2.5	5.0
I-methylate		2.5	3.0	0.5	2.5
J		1.5	2.0	0.3	0.6
K		0.1	0.1	0.1	0.4
K-triacetate		0.06	0.1	0.1	0.1
L		0.6	0.7	0.4	0.6
L-triacetate		1.5	1.5	1.5	1.5
M		0.08	0.09	0.04	0.09
M-triacetate		0.6	0.7	0.5	2.0
N-triacetate		0.09	0.2	0.06	0.1
	Adriamycin	0.15	1.2	0.15	3.0
	Verapamil	10.0	10.0	9.0	4.0
	PSC833	0.02	0.05	0.04	0.1

- 10 -

TABLE III

Adriamycin Toxicity to Resistant Cells (MDR) in Presence or in Absence  
of Reversing Agents (R.A.)  
Gain of Sensitivity (G.S.) =  $IC_{50} \text{ Control} / IC_{50} \text{ R.A.}$

REVERSING AGENT (R.A.)	R.A. ( $\mu\text{g/ml}$ )	Mouse Lymphoma (MDR)		Chinese Hamster Ovary (MDR)	
		P-388/SCHABEL $IC_{50}$ ( $\mu\text{g/ml}$ )	G.S.	CHOC5 $IC_{50}$ ( $\mu\text{g/ml}$ )	G.S.
CONTROL (Adriamycin only)	0	1.2		3.0	
Verapamil (Standard)	1	0.15	8.0	0.2	15.0
	3	0.06	20.0	0.1	30.0
Lamellarin A	0.1	0.7	1.7	2.0	1.5
	0.3	0.2	6.0	1.0	3.0
Lamellarin B	1	0.3	4.0	0.7	4.3
	3	0.15	8.0	0.003	1,000
Lamellarin D-triacetate	0.01	0.7	1.7	2.5	1.2
	0.03	0.12	10.0	0.003	1,000
Lamellarin I	0.1	0.4	3.0	0.6	5.0
	0.3	0.15	8.0	0.15	20.0
	1	0.04	30.0	0.01	300
Lamellarin I-acetate	1	0.25	4.8	0.4	7.5
	3	0.07	17.1	0.08	37.5
Lamellarin I-Methylate	0.3	0.2	6.0	0.4	7.5
	1	0.04	30.0	0.12	25.0
Lamellarin J	0.1	0.9	1.3	2.5	1.2
	0.3	0.7	1.7	2.0	1.5
Lamellarin K	0.03	0.9	1.3	2.0	1.5
	0.1	0.3	4.0	0.9	3.3
Lamellarin K-triacetate	0.03	0.4	3.0	1.0	3.0
Lamellarin L	0.1	0.4	3.0	2.5	1.2
	0.3	0.003	400	2.0	1.5
Lamellarin L-triacetate	0.3	0.2	6.0	0.1	30.0
	1	<0.003	>400	0.003	1,000
Lamellarin M	0.03	1.0	1.2	1.2	2.5
Lamellarin M-triacetate	0.1	0.6	2.0	2.0	1.5
	0.3	0.4	3.0	1.0	3.0
Lamellarin N-triacetate	0.01	0.8	1.5	2.0	1.5
	0.03	0.3	4.0	0.4	7.5

- 11 -

**Determination of MDR Activity:**

Sensitive and MDR cells were maintained, in logarithmic phase of growth, in Eagle's Minimum Essential Medium, with Earle's Balanced  
5 Salts, with non-essential amino acids, with 2.0 mM L-glutamine, without sodium bicarbonate (EMEM/nea), supplemented with 5% Fetal Calf Serum (FCS),  $10^{-2}$ M sodium bicarbonate and 0.1 g/l penicillin G + 0.1 g/l streptomycin sulfate.

10 *In Vitro* cell cytotoxicity was determined using the 3-[4,5-dimethylthiazol-2-yl]-2,5-dipheniltetrazolium bromide (MTT), from Sigma Ref:M-2128, for quantitative measurement of cell growth and viability. See, T. Mosmann, "Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays," *Journal*  
15 *of Immunological Methods*, **65**: 55-63 (1983).

The tumor cell lines employed have been: P-388 (ATCC CCL 46), suspension culture of a lymphoid neoplasm from a DBA/2 mouse and its corresponding MDR cell line P-388/SCHABEL; CHOB1 (ATCC CCL 16),  
20 monolayer culture of chinese hamster ovary and its corresponding MDR cell line CHOC5. See, Rauscher III, et al., "Characterization of Auromycin-Resistant Hamster Cell Mutants that Display a Multidrug Resistance Phenotype," *Molecular Pharmacology*, **38**: 198-206 (1990).

25 This form of assay employs 96-well cell culture plates 99 mm diameter. Cells were seeded into wells at  $1 \times 10^3$  cells per well in 100  $\mu$ l aliquots of EMEM 5% FCS containing different concentrations of the corresponding lamellarins and other compounds (standards) to be tested. Two separate sets of cultures without drugs were seeded, one as control  
30 of growth to ensure that cells remained in exponential phase of growth,

- 12 -

and another without cells as control of medium. All determinations were carried out in duplicate.

5 After three days of incubation at 37°C, 10% CO<sub>2</sub> in a 98% humid atmosphere, 150 µg of MTT is added to each well in 50 µl aliquots of assay medium. Plates were incubated for an additional 4 hours and 100 µl aliquots of isopropanol were added to each well. The broad absorption spectrum for the isopropanol solution of this crystal is optimal at 570 nm. Optical density values were obtained with the help of a  
10 Dynatech microplate reader and the results of the assay were used to generate graphics from which IC<sub>50</sub> was calculated; i.e., wherein the IC<sub>50</sub> is the test concentration which produces 50% cell growth inhibition.

#### Other Biological Properties:

15

The lamellarin compounds also possess immunomodulation activity, and will thus be useful as immunomodulator compounds. Immunomodulator compounds and compositions, as the name implies, are useful for modulating or regulating immunological functions in warm  
20 blooded animals. Immunomodulators may be immunostimulants for building up immunities to or initiate healing of certain diseases and disorders. Conversely, they may be immunoinhibitors or immunosuppressors for preventing undesirable immune reactions of the body to foreign materials and autoimmune diseases.

25

Immunomodulators have been found to be useful for treating systemic autoimmune diseases, such as lupus erythematosus, as well as immunodeficiency diseases. Further, immunomodulators may be useful for immunotherapy of cancer or to prevent rejections of foreign organs  
30 or other tissues in transplants, e.g., kidney, heart or bone marrow.

- 13 -

Lamellarins I, K and L all exhibit comparable and significant cytotoxicity against P388 and A549 cell lines in culture ( $IC_{50} \approx 0.25 \mu\text{g/ml}$  against each cell line). Lamellarins K and L also exhibited moderate immunomodulatory activity (LcV:MLR 147 and 98 respectively), and as such, have specific art recognized utilities related thereto.

As shown below in Table IV, the lamellarin compounds M, J and N triacetate, have surprisingly been found to have *in vitro* antitumor activities which are significantly better, particularly against A549 cells, than the lamellarins I, K and L.

As shown below in Table V, the *in vivo* antitumor activity of lamellarin K is consistent with the *in vitro* activity demonstrated above. Based upon these data, it is believed that the lamellarin compounds disclosed herein will be useful as antitumor compounds, particularly against the following tumor cell types; leukemia (P388), human lung carcinoma (A549), human colon carcinoma (HT-29), human melanoma (MEL-28).

20

25

30

- 14 -

TABLE IV

**Lamellarin *In Vitro* Activity vs. Tumor Cell lines (IC<sub>50</sub>  $\mu$ g/mL)**

	P388	A549	HT-29	MEL-28
Lamellarin I	1	1	2	--
Lamellarin K	0.25	0.25	1	--
Lamellarin L	0.25	0.25	2	--
Lamellarin M	0.05	0.025	0.5	0.5
Lamellarin J	0.1	0.025	2.5	2.5
Lamellarin N triacetate	0.1	0.012	5	5



TABLE V  
*In Vivo* Anti-Tumor Activity vs. P388 Lymphocytic Leukemia

compound	Dose (mg/kg) inject. Total	Schedule & Route	Body Weight Day 0 (gm) + SD	Body Weight Day 5 (gm) + SD	Body Weight Change (gm) Day 5	Mean Survival Time	% T/C
LAM K (7)	15.000 75.000	QD 1-5 ip	19.5 $\pm$ 0.5	20.7 $\pm$ 0.5	1.2	28.3 $\pm$ 5.9	> 135.0°

P388 cells ( $10^6$ ) were implanted i.p. into female CD2F1 mice on day 0. Compounds were administered in saline vehicle i.p. days 1-5 or 1-9 in a volume of 0.5 mL/animal. Mice were weighed on days 0 and 5 and deaths were recorded daily.

\* Significant activity (moderate): T/C  $\geq$  125%

- 16 -

The present invention has been described in detail, including the preferred embodiments thereof. However, it will be appreciated that those skilled in the art, upon consideration of the present disclosure, may make modifications and/or improvements on this invention and still  
5 be within the scope and spirit of this invention as set forth in the following claims.

10

15

20

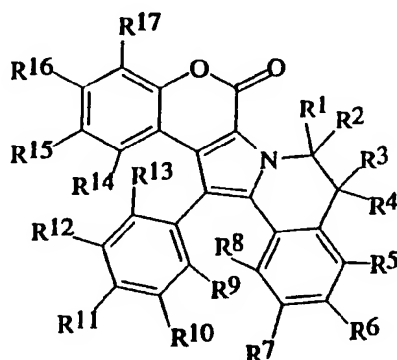
25

30

**WHAT IS CLAIMED IS:**

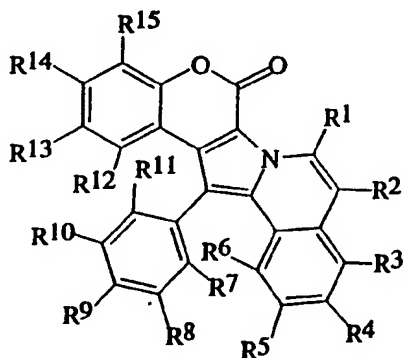
1. A method of treating multidrug resistant tumors in mammals, comprising administering to a patient in need of such treatment, an effective MDR inhibiting amount of an anti-MDR lamellarin compound.

2. The method of claim 1, wherein the anti-MDR lamellarin compound has the following formula:



wherein each of R<sup>1</sup> to R<sup>17</sup> may be the same or may be different and are selected from the group consisting of -H, -OH, -Me, -Et, -Pro, -OMe, -COMe, and -OCOMe.

3. The method of claim 1, wherein the anti-MDR lamellarin compound has the following formula:

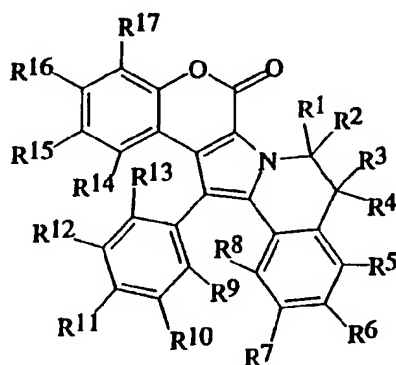


- 18 -

wherein each of  $R^1$  to  $R^{15}$  may be the same or may be different and are selected from the group consisting of -H, -OH, -Me, -Et, -Pro, -OMe, -COMe, and -OCOMe.

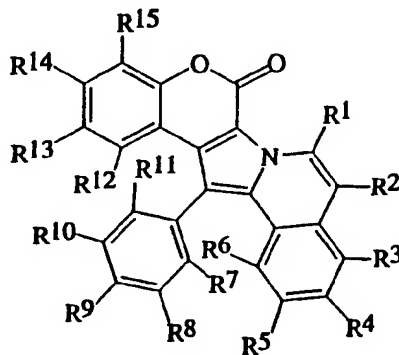
4. A method of treating multidrug resistant tumors in mammals, comprising administering to a patient in need of such treatment, an effective MDR cell cytotoxic amount of an anti-MDR lamellarin compound.

5. The method of claim 4, wherein the anti-MDR lamellarin compound has the following formula:



wherein each of  $R^1$  to  $R^{17}$  may be the same or may be different and are selected from the group consisting of -H, -OH, -Me, -Et, -Pro, -OMe, -COMe, and -OCOMe.

6. The method of claim 4, wherein the anti-MDR lamellarin compound has the following formula:



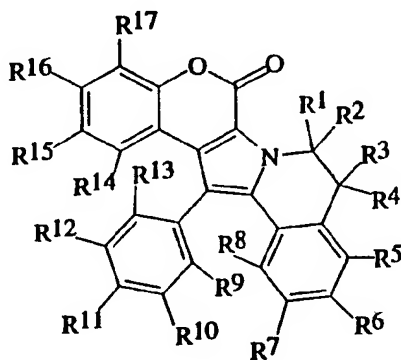
- 19 -

wherein each of  $R^1$  to  $R^{15}$  may be the same or may be different and are selected from the group consisting of -H, -OH, -Me, -Et, -Pro, -OMe, -COMe, and -OCOMe.

7. A pharmaceutical composition comprising one or more anti-MDR lamellarin compounds in combination with one or more MDR affected antitumor drugs.

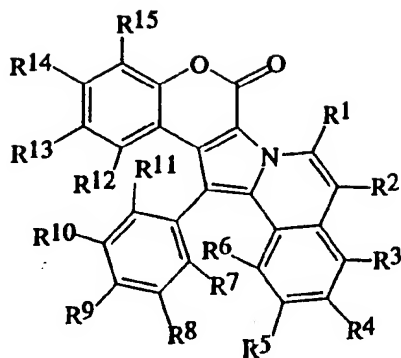
8. The pharmaceutical composition of claim 7, wherein the MDR affected antitumor drug is selected from the group consisting of vinblastine, vincristine, etoposide, teniposide, doxorubicin (adriamycin), daunorubicin, plicamycin (mithramycin) and actinomycin D.

9. The pharmaceutical composition of claim 7, wherein the anti-MDR lamellarin compound has the following formula:



wherein each of  $R^1$  to  $R^{17}$  may be the same or may be different and are selected from the group consisting of -H, -OH, -Me, -Et, -Pro, -OMe, -COMe, and -OCOMe.

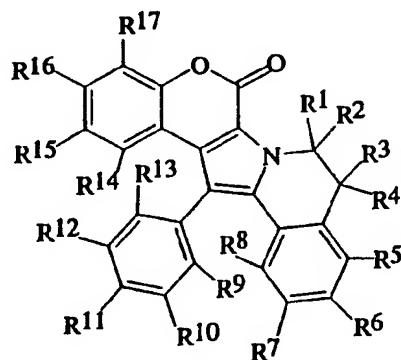
10. The pharmaceutical composition of claim 7, wherein the anti-MDR lamellarin compound has the following formula:



wherein each of  $R^1$  to  $R^{15}$  may be the same or may be different and are selected from the group consisting of -H, -OH, -Me, -Et, -Pro, -OMe, -COMe, and -OCOMe.

11. A method of improving the antitumor chemotherapeutic effect of MDR affected drugs in patients in need of such treatment, comprising coadministering the MDR affected antitumor drug with an effective amount of an anti-MDR lamellarin compound.

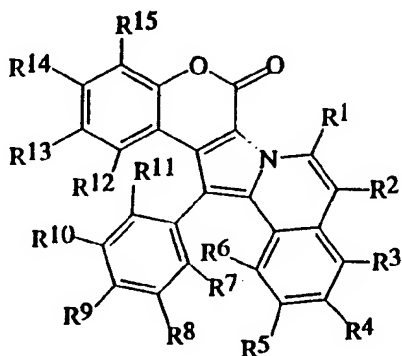
12. The method of claim 11, wherein the anti-MDR lamellarin compound has the following formula:



- 21 -

wherein each of R<sup>1</sup> to R<sup>17</sup> may be the same or may be different and are selected from the group consisting of -H, -OH, -Me, -Et, -Pro, -OMe, -COMe, and -OCOMe.

13. The method of claim 11, wherein the anti-MDR lamellarin compound has the following formula:



wherein each of R<sup>1</sup> to R<sup>15</sup> may be the same or may be different and are selected from the group consisting of -H, -OH, -Me, -Et, -Pro, -OMe, -COMe, and -OCOMe.

14. The method of claim 11, wherein the MDR affected antitumor drug and the anti-MDR lamellarin compound are administered simultaneously.

15. The method of claim 11, wherein the MDR affected antitumor drug and the anti-MDR lamellarin compound are administered sequentially.

## INTERNATIONAL SEARCH REPORT

Inter. Jnal Application No

PCT/IB 96/00742

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K31/435

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 107, 1985, USA, pages 5492-5495, XP000602056 ANDERSEN ET AL: "Metabolites of the Marine Prosobranch Mollusc Lamellaria sp." cited in the application see the whole document ---	1-15
A	JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 53, 1988, USA, pages 4570-4574, XP000602058 LINDQUIST ET AL: "New Alkaloids of the Lamellarin class from the marine Ascidian Didemnum chartaceum" cited in the application see the whole document --- -/--	1-15

☒ Further documents are listed in the continuation of box C.☐ Patent family members are listed in annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- \*&\* document member of the same patent family

Date of the actual completion of the international search

24 September 1996

Date of mailing of the international search report

09.10.96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax (+31-70) 340-3016

Authorized officer

Herrera, S



# INTERNATIONAL SEARCH REPORT

Inter. onal Application No  
PCT/IB 96/00742

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>AUSTRALIAN JOURNAL OF CHEMISTRY, vol. 46, no. 4, 1993, AUSTRALIA, pages 489-501, XP000602402 CARROLL ET AL: "Studies of Australian Ascidians. I, Six New Lamellarin-Class Alkaloids from a Colonial Ascidian, Didemnum sp." cited in the application see the whole document -----</p>	1-15